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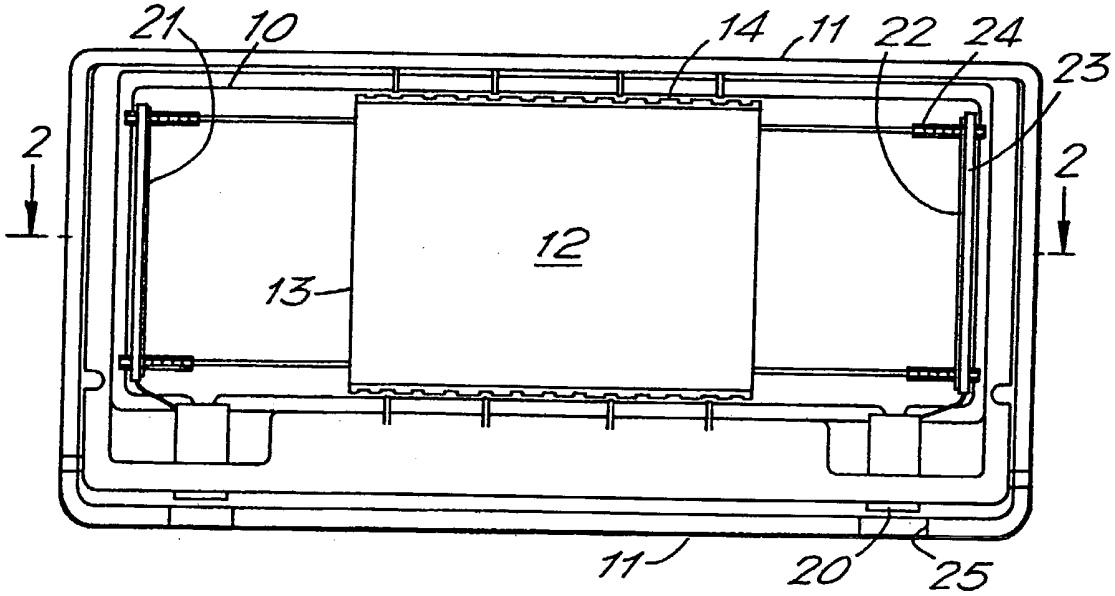
(54) Title: ANALYSIS OF BIOLOGICAL MOLECULE SAMPLES



(57) Abstract

Apparatus for electrophoresis of a biological molecule sample comprises a tank (10), with electrodes (21, 22) at opposite sides and, between the electrodes, means for receiving a stack of at least three gel trays (12) lying parallel one above the other. Methods of blotting the electrophorised gel are disclosed.

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Analysis of biological molecule samples

This invention concerns electrophoresis and analysis of biological molecule samples, and apparatus and methods for this.

The usual method of analysing a biological molecule sample has a number of stages. Very broadly, these may include: enzyme reaction with a biological molecule sample in an aqueous solution in a microtitre tube; transfer of the sample to an agarose gel; electrophoresis of the gel in an electrolyte tank to separate the molecules along the length of gel according to their relative size; blotting of the gel to transfer and stabilise the separated samples; and analysis of the blot. These stages are currently poorly coordinated, and individually are very time consuming and subject to inaccuracy due to lack of definition of the blots.

The invention aims to provide new apparatus and methods which remedy or at least reduce the defects of the existing system.

Electrophoresis achieves a migration of molecules by different amounts from an initial position in the gel, depending on the length of the molecules. The distances travelled by all molecules depends on the electrical voltage and current, temperature, and conditions in the electrophoresis tank. The accuracy of the subsequent analysis of blots made from the gel depends upon the sharpness of the set of bands formed by the molecules, and the straightness of each individual band. Major problems in these respects arise because temperature rises in the tank cause distortions of the gel and

therefore of the individual bands, and the current lengthy capillary blotting process causes loss of sharpness.

According to one feature of the invention, there is provided apparatus for electrophoresis of a biological molecule sample, comprising a tank with electrodes at two opposite sides and, between the electrodes, means for receiving a stack of at least three gel trays lying parallel one above the other.

Such an apparatus requires a considerable depth, and therefore volume, of electrolyte compared with currently used electrophoresis tanks. It has been found unexpectedly that this tends to prevent distortion of the gel and of the bands because the greater thermal mass of the electrolyte suffers less temperature rise due to the current flow. An equivalent increase in the volume of electrolyte in a tank for a single gel tray would be quite uneconomic.

Further, the treatment of three gels at once in a single apparatus is very much more economical and allows the whole contents of standard microtitre plates to be handled in a single run, as will be described.

According to a further feature of the invention, there is provided a method of blotting biological molecule samples from a gel to a carrier membrane, comprising placing a carrier membrane against a surface of a gel, inserting both into a centrifuge membrane outwards, and running the centrifuge so that the sample migrates from the gel to the membrane. Preferably, the gel is left in the tray in which it has been electrophoresed. This

method is to be compared with the previously used method of capillary blotting, in which the gel is removed from its tray, a membrane and towels are placed on top of the web and a heavy weight is placed on top of the towels to draw the moisture and the samples upward. Vacuum blotting, the other alternative, uses special and expensive apparatus. Using the new method, the process can be effected in less than 30 minutes compared with many hours for normal capillary blotting.

If a centrifuge is not available, the invention proposes a yet further method of blotting biological molecule samples from a gel to a carrier membrane, comprising placing a membrane over a gel in a tray, placing a spongy layer over the membrane, placing a rigid sheet over the spongy layer, and applying a compression force between the rigid sheet and the gel tray. This method effects transfer in less than 8 hours.

Not only are these new processes advantageous because they are quicker, but they produce a sharper results. Longer timespans allow the molecules to diffuse laterally and cause the bands to lose sharpness. This does not happen with the new methods of blotting.

In order that the invention shall be clearly understood an exemplary embodiment thereof will now be described with reference to the accompanying drawing, in which:

Fig.1 shows the plan view of an electrophoresis tank according to the invention; and

Fig.2 shows a section on 2-2 in Fig.1.

The tank is in the form of a rectangular box 10 about 12cm by 25cm. It has a hinged lid 11, made of transparent plastic so that the progress of a process can be monitored visually. Its depth is rather greater than would be normal for an electrophoresis tank.

Within the box 10 there are stacked three gel trays 12. The gel trays stand upon another. Each gel tray comprises a base 13 and two side portions 14. The side portions are grooved and abut the sides of the box. Four webs 15 protrude from each side of the box 10, and ensure the gel trays are held firmly in position in the tank.

The lid 11 is pivotable about fixed pegs 16. Two electrical sockets, 20, one positive and one negative, are mounted in the back wall of the tank 10 near the top edge. The sockets are connected to a straight platinum wire which extends across the opposite end surfaces of the tank to form an anode 21 and a cathode 22. The anode and cathode are each mounted on an electrode plate 23 which is held in a stepped holder 24. The anode and cathode are each positioned level with the mid-position of the gel trays held in the tank. The electrical sockets 20 lie within a lip at the back of the lid 11. Arched cut outs 25 permit access to the sockets for the insertion of supply leads (not shown). However, with the supply leads in position, the lid cannot be opened, and conversely the supply leads cannot be inserted into the sockets with the lid in the open position. There is thus no chance of an operator inadvertently receiving a shock from the electrodes or from an electrolyte in the tank.

The positioning of the electrodes level with the mid-position of the trays ensures in practice that identical results are obtained with all three trays. The path differences for the electrolytic current are negligible. This is important when comparisons need to be made between samples in different trays.

Because three trays are stacked, the tank is considerably deeper than conventional electrophoresis tanks and contains a considerably larger volume of electrolyte. Consequently, the thermal mass of the tank is much higher and any temperature rise caused by the electric current produces only a negligible increase in temperature of the whole tank. Thus, the results achieved by the electrophoresis in separating different molecules is sharper and the analysis can be more accurate.

The number of gel trays in the tank can be varied to suit differing operating requirements. A preferred modification is to use four gel trays. To vary the number of trays in the tank the electrode plates 23 are repositioned in the stepped holders 24 so that the anode and cathode remain level with the mid-position of the trays. The stepped holders are provided with positions for use with two to four gel trays. If a single gel tray is used the positions of the anode and cathode are not crucial.

In a modification, the gel trays can be supported in a stack by structures within the tank. The structures are formed along each of the longer sides which forms a set of shoulders or ledges for receiving gel trays. The structure on each side may comprise a set of three

elongate bars, each of which carries retaining pegs and bores. The bars are located between themselves and within the tank by the pegs, but the structures can be removed for cleaning of the tank. Equally, the bars are formed so as to retain the gel trays in spaces relationship one above the other.

As a means of unifying the analysis of biological molecule samples, certain standard dimensions are preferably adhered to in the production of the tank and the ancillary apparatus. In each gel tray are formed an array of depressions or sample wells, into which the material to be analysed is placed. The gel trays and sample wells are made compatible with direct loading of multiple samples from standard microtitre plates. This allows large sample numbers to be processed rapidly, minimises loading error and facilitates consistent repeat loadings of the same sample set. The gel tray is also based on the standard size of a 96-well microtitre dish (126mm x 86mm, nominal) which means that nucleic acids can be transferred to nitrocellulose or nylon membranes by centrifugal blotting as an alternative to conventional capillary or vacuum transfer.

The sample wells in the gel trays are formed using slot formers. The slot formers engage the grooves in the side portions 14 of the gel trays. The slot formers or combs are constructed to give 8,16 or 24 sample wells, with an additional small slot on each side for loading marker tracks. The sample wells are spaced to align with the tips on a standard 8- channel microtitre pippette. Alternate wells are loaded when using the 16 track comb and every third track is loaded each time with the 24 track comb. Each gel slab on a gel tray is cast using 1

to 4 sample combs, resulting in an effective gel length of from 120mm down to 30mm. Thus, as many as 96 samples can be electrophoresed per gel. The tank is designed so that up to four gel trays can be stacked and run simultaneously. Thus, up to 72 full length or 288 short track samples can be electrophoresed simultaneously from a single power outlet. The maximum sample volumes are determined by the amount of gel slab (typically 50ml, giving a 5mm thick slab and 4mm sample depth), the width of the sample well (7.5mm per 8 track comb, 3.0mm per 16 track comb and 1.5mm per 24 track comb) and the comb thickness (1.0 or 1.5mm). 1,2 or 4 slot combs are also available for running large volume preparative gels. The system is equally suited to the electrophoresis of genomic, cosmid, lambda or plasmid DNA's, as well as the products of Polymerase Chain Reactions and RNA samples.

Once samples have been electrophoresed they need to be treated prior to blotting. Usually gels are removed from the tank to do this. The tank however, allows treatment to take place in-situ in the tank. Using two ports at either end of the tank (not shown) on opposite sides, one at a high point and one at a low point, the contents of the tank are continually changed, thus actively treating the gels.

After electrophoretic resolution, the gel is retained within the gel casting tray and processed for blotting by depurination, denaturation and neutralisation, as required. The end slots are sealed, eg with PVC tape or a silicon rubber clamp.

Two blotting methods are then possible. In the first,

the gel is over laid with the chosen membrane support, followed by an absorbant (alkali resistant, synthetic) sponge. The sponge has a rigid housing or lid having microtitre plate format such that the edges match exactly with those of the gel tray. The thickness of sponge is such that moderate force is required to seal the lid and tray. Contact is maintained throughout the transfer period by sealing around the matched edges with a clip or PVC tape device. This allows a more or less specific and evenly spread pressure to be applied to squeeze the gel against the membrane.

The effect is to promote capillary transfer while minimising the extent of gel collapse which follows from methods utilising an unrestricted weight on an absorbant stack and which inhibits nucleic acid transfer out of the gel. Maximum transfer is typically achieved in 4-6 hrs, with a sensitivity equal to or in excess of conventional capillary transfer methods. However, by this method the transfer is achieved with an absolute minimum of gel handling, consumption of reagents and laboratory space. In particular, the gel remains in its tray.

In the second method, similar to that for capillary transfer just described except that the thickness of sponge is such that the edges of the lid and tray are sealed with minimum compression. The edges are sealed with PVC tape, or similar, and the gel/membrane/sponge sandwich is placed in a centrifuge on a rotor designed to spin microtitre plates, with the gel towards the centre, so that the centrifugal force will drive the DNA out of the gel and onto the membrane. Efficient DNA transfer is typically achieved in 20 min. at 400rpm.

Sensitivity is comparable to conventional capillary transfer methods.

Both methods are simple, rapid and efficient and involve a minimum of gel handling, consumption of laboratory reagents, equipment and space. The methods are fully proven for transfer of simple and complex DNA's, including PCR products, bacterial and yeast cloned DNA's and eukaryotic genomic DNA's. The methods can be adapted to effect RNA transfer.

Claims

1. Apparatus for electrophoresis of a biological molecule sample, comprising a tank with electrodes at two opposite sides and, between the electrodes, means for receiving a stack of at least three gel trays lying parallel one above the other.

2. An apparatus according to claim 1, wherein both electrodes are positioned level with the middle of said stack of gel trays.

3. An apparatus according to claims 1 or 2, wherein said means comprise a plurality of protruding members formed on the sides of the tank, upon which said trays rest.

4. A system for the electrophoresis of biological molecule samples, comprising an apparatus according to any preceding claim, and at least three gel trays, each gel tray comprising a base having a layer of agarose gel formed thereon, the layer of gel having a plurality of wells formed therein for receiving samples of biological molecular material.

5. A system according to claim 4, wherein said plurality of wells are arranged in an array which is spaced to align with a standard 8-channel microtitre pippette.

6. A system according to any preceding claim, wherein the gel trays further comprise side members formed perpendicular to the base.

7. A system according to claim 6, wherein said stack of gel trays is formed by resting the base of the lowermost tray on the floor of the tank, and standing the bases of the other trays on the side members of the lowermost adjacent tray in the stack.

8. A method of blotting biological molecule samples from gel to a carrier membrane, comprising placing a carrier membrane against a surface of a gel, inserting both into a centrifuge web outwards, and running the centrifuge so that the sample migrates from the gel to the web.

9. A method of blotting biological molecule samples from a gel to a carrier membrane, comprising placing a membrane over a gel in a tray, placing a spongy layer over the membrane, placing a rigid sheet over the spongy layer, and applying a compression force between the rigid sheet and the gel tray.

10. A method according to claims 8 or 9, wherein the biological molecules are electrophoresed in a tray prior to blotting, and the blotting is carried out with the gel left in the tray in which electrophoresis took place.

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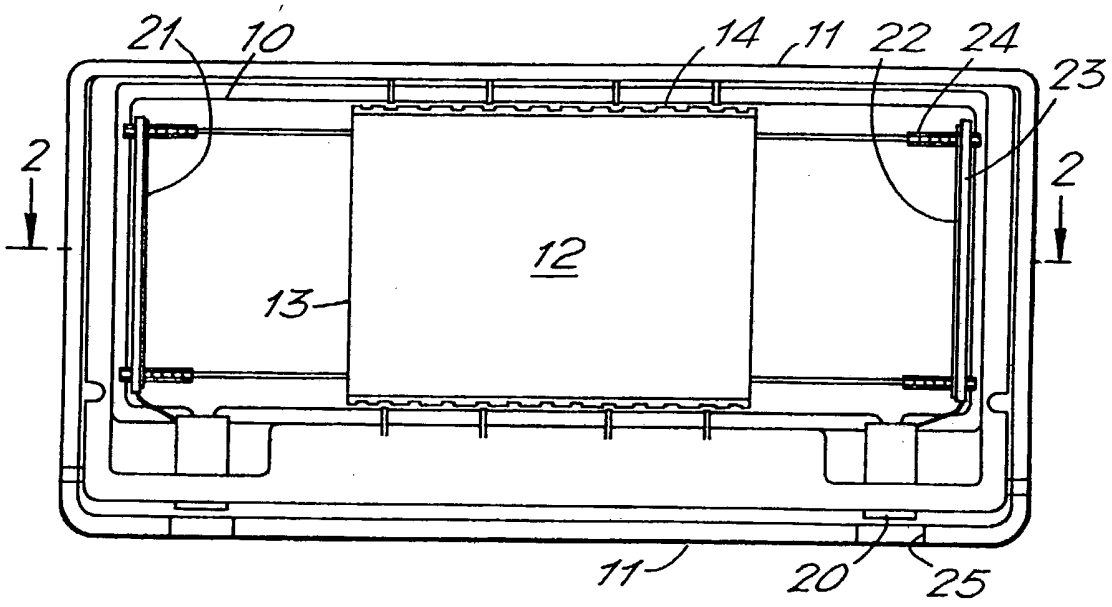
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FIG.1.

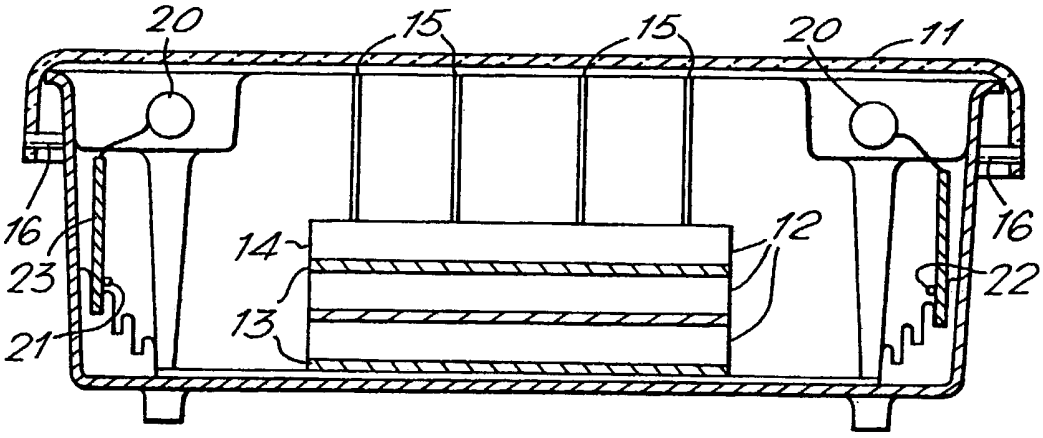
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FIG.2.

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 91/00023

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC5: G 01 N 27/447, 27/26

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
IPC5	G 01 N; B 01 D

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category *	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
X	EP, A2, 0110274 (DEUTSCHES KREBSFORSCHUNGSZENTRUM) 13 June 1984, see abstract; claim 7	1
Y		4
	--	
Y	EP, A1, 0236153 (ONCOR, INC) 9 September 1987, see abstract	4
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X	EP, A2, 0380357 (WHITEHEAD INSTITUTE FOR BIOMEDICAL RESEARCH) 1 August 1990, see abstract	8
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* Special categories of cited documents: [10]

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IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
2nd April 1991	- 3. 05. 91
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	M. PEIS M. Peis

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
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A	GB, A, 2147609 (PUBLIC HEALTH LABORATORY SERVICE BOARD (UNITED KINGDOM)) 15 May 1985, see the whole document	1-10
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A	EP, A2, 0300924 (ONCOR, INC) 25 January 1989, see the whole document	1-10

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.PCT/GB 91/00023

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This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on 28/02/91
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